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Semidominant Suppressors of Srs2 Helicase Mutations of *Saccharomyces cerevisiae* Map in the *RAD51* Gene, Whose Sequence Predicts a Protein with Similarities to Procaryotic RecA Proteins

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Eleven suppressors of the radiation sensitivity of *Saccharomyces cerevisiae* diploids lacking the Srs2 helicase were analyzed and found to contain codominant mutations in the *RAD51* gene known to be involved in recombinational repair and in genetic recombination. These mutant alleles confer an almost complete block in recombinational repair, as does deletion of *RAD51*, but heterozygous mutant alleles suppress the defects of *srs2::LEU2* cells and are semidominant in Srs2$^+$ cells. The results of this study are interpreted to mean that wild-type Rad51 protein binds to single-stranded DNA and that the semidominant mutations do not prevent this binding. The cloning and sequencing of *RAD51* indicated that the gene encodes a predicted 400-amino-acid protein with a molecular mass of 43 kDa. Sequence comparisons revealed homologies to domains of *Escherichia coli* RecA protein predicted to be involved in DNA binding, ATP binding, and ATP hydrolysis. The expression of *RAD51*, measured with a *RAD51-lacZ* gene fusion, was found to be UV- and γ-ray-inducible, with dose-dependent responses.

In *Saccharomyces cerevisiae*, the different DNA repair mechanisms are controlled by genes that have been classified into epistasis groups, according to the synergistic, additive, or epistatic interactions of the mutant genes (for reviews, see references 14 and 19). Synergistic interactions were interpreted to reflect the existence of different repair pathways having a common substrate (13, 15). In a mutant, the repair metabolism may be arrested at a step involving a compound that may or may not be a substrate for an alternative pathway. This, in some cases, may explain some of the diversity in the phenotypes conferred by mutations in different genes in the same epistatic group. The study of interactions between mutations, either by the construction of double mutants or by the isolation of suppressors of mutant phenotypes, is a way to explore the pathways and their possible connections.

The *SRS2* gene (also *HPR5* and *RADH* [42]) of *S. cerevisiae* encodes a DNA helicase involved in DNA repair (1), its helicase activity having recently been demonstrated by in vitro assays (27). All of the known *srs2* mutants are partial suppressors of the high radiation sensitivity of *rad18* and *rad6* mutants (1, 2, 30, 42, 47), both of which have impaired error-prone repair processes (8, 29). A subset of the *srs2* mutations, including the deletion mutations, confer to haploid cells a depressed UV-induced mutagenesis and a sensitivity to the lethal effect of UV. However, only cells treated in the G_1 mitotic phase are sensitive to UV, while cells in G_2 show a UV resistance dependent on the *RAD50* gene, needed for recombinational repair involving sister chromatids (16). These results led us to conclude that the lack of *SRS2* results in a channelling of the metabolism of potentially mutagenic lesions into a recombinational pathway.

An unexpected property of *srs2::LEU2* cells was the radiation sensitivity of homozygous diploids, as the channelling yields to lethal events of recombination if homologous chromosomes are involved. To approach this question, we have studied suppressors of this diploid sensitivity. The genetic characterization of a number of suppressors indicated that they all contained a codominant mutation in a single gene that turned out to be *RAD51*. This gene, cloned several years ago by Calderon et al. (7), is known to control recombinational repair and different aspects of genetic recombination (36, 45; for a review, see reference 14). Neither a *rad51* genomic deletion nor *rad51* mutations isolated on the basis of their X-ray sensitivity are, when heterozygous, suppressors of the radiation sensitivity of *srs2::LEU2* diploids.

The first part of this communication deals with the interaction of mutations in *RAD51* and *SRS2* genes. We show that the channelling to recombination observed in *srs2* mutants following UV irradiation is likely due to the binding of recombinational proteins to single-stranded DNA. Since all the codominant suppressors of the radiation sensitivity of *srs2* diploids were *RAD51* mutants, it is believed that the Rad51 protein plays a central role in the initiation of recombination events, which could well correspond to the binding of the protein to single-stranded DNA.

In the second part of this article, we report the sequences of the *RAD51* gene and of the putative Rad51 protein which shares homologies with the procaryotic RecA protein. We also show that the *RAD51* gene is induced in response to radiation.

MATERIALS AND METHODS

Strains, media, growth conditions, and transformation methods. The yeast strains are listed in Table 1. The *rad51-1* mutation was initially isolated by Nakai and Matsumoto (37). The isogenic series of strains were constructed by transforming haploid strain FF18733 with a replicative plasmid containing *HO* in order to induce the mating-type switch and to

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TABLE 1. Yeast strains

Cell type and strain	Relevant genotype
Isogenic haploids	
FF18733	**a** *leu2-3,112 trp1-289 ura3-52 lys1-1 his7-2*
FF18734	α leu2-3,112 trp1-289 ura3-52 lys1-1 his7-2
FF18744	**a** *leu2-3,112d dtrp1-289 ura3-52 lys1-1 his7-2 srs2::LEU2*
FF18745	α *leu2-3,112 trp1-289 jura3-52 lys1-1 his7-2 srs2::LEU2*
FF18816	**a** *leu2-3,112 trp1-289 ura3-52 lys1-1 his7-2 rad51-10*
FF18818	α leu2-3,112 trp1-289 ura3-52 lys1-1 his7-2 rad51-10
FF18958	**a** *leu 2-3,112 trp1-289 ura3-52 lys1-1 his7-2 rad51::URA3*
FF18959	α *leu2-3,112 trp1-289 ura3-52 lys1-1 his7-2 rad51::URA3*
FF18785	**a** *leu2-3,112d trp1-289 ura3-52 lys1-1 his7-2 srs2::LEU2 rad51-10*
FF18787	α *leu2-3,112 trp1-289 ura3-52 lys1-1 his7-2 srs2::LEU2 rad51-10*
FF18961	**a** *leu2-3,112 trp1-289 ura3-52 lys1-1 his7-2 srs2::LEU2 rad51::URA3*
FF18962	α *leu2-3,112 trp1-289 ura3-52 lys1-1 his7-2 srs2::LEU2 rad51::URA3*
Isogenic a/α diploids derived from the isogenic haploids	
FF18735 (FF18733 × FF18734)	*SRS2/SRS2 RAD51/RAD51*
FF18749 (FF18744 × FF18745)	*srs2::LEU2/srs2::LEU2 RAD51/RAD51*
FF18982 (FF18816 × FF18818)	*SRS2/SRS2 rad51-10/rad51-10*
FF18960 (FF18958 × FF18959)	*SRS2/SRS2 rad51::URA3/rad51::URA3*
FF18981 (FF18816 × FF18734)	*SRS2/SRS2 rad51-10/RAD51*
FF181048 (FF18958 × FF18734)	*SRS2/SRS2 rad51::URA3/RAD51*
FF 18789 (FF18785 × FF18787)	*srs2::LEU2/srs2::LEU2 rad51-10/rad51-10*
FF18963 (FF18961 × FF18962)	*srs2::LEU2/srs2::LEU2 rad51::URA3/rad51::URA3*
FF18952 (FF18961 × FF18745)	*srs2::LEU2/srs2::LEU2 rad51::URA3/RAD51*
FF18784	*srs2::LEU2/srs2::LEU2 rad51-10/RAD51*
Other strains	
FF181002	**a** *leu2-3,112 trp1-289 lys1 lys2 his7-1 RAD51*
	α *LEU2 TRP1 LYS1 LYS2 his7-2 RAD51*
FF181005	**a** *leu2-3,112 trp1-289 lys1 lys2 his7-1 rad51::URA3*
	α *LEU2 TRP1 LYS1 LYS2 his7-2 rad51::URA3*
FF181173	**a** *leu2-3,112 trp1-289 lys1 lys2 his7-1 rad51::URA3*
	α *LEU2 TRP1 LYS1 LYS2 his7-2 RAD51*
FF181179	**a** *leu2-3,112 trp1-289 ura3-52 lys1 tyr1 his7-1 rad51-10*
	α *leu2-3,112 trp1-289 ura3-52 LYS1 TYR1 his7-1 RAD51*
FF181056	**a** *leu2-3,112 trp1-289 ura3-52 lys1 tyr1 his7-1 rad51-10*
	α *leu2-3,112 trp1-289 ura3-52 LYS1 TYR1 his7-1 rad51-10*

[a] This strain kindly provided by D. H. Hawthorne.

recover diploids which were sporulated to isolate haploids of both mating types. Genomic deletions of *RAD51* or *SRS2* were done by the one-step gene disruption method (44) using a *rad51::URA3* plasmid (this study) or a *srs2::LEU2* plasmid constructed in our laboratory. *Escherichia coli* strains were HB101, TG1, and JM101 (JM101 was used for phage production). For yeast cells, the growth (YPD), minimal (SD), and sporulation (SM) media were as described by Sherman et al. (48). The YPD-MMS medium consisted of YPD medium containing 0.01% methyl methane sulfonate (MMS) (Sigma). For selection of *can1* mutants, 20 μg of canavanine (Sigma) per ml was added to SD medium supplemented with the desired elements. LB and M9 media (34) were used for bacteria. Yeast cells were grown at 30°C. The liquid cultures were aerated by vigorous agitation. Transformation of yeast cells was done with lithium acetate (23), and transformation of bacteria was done by the method of Maniatis et al. (34).

Irradiation. Cells growing exponentially (1×10^7 to 2×10^7/ml) in liquid YPD medium were washed by centrifugation in saline (0.9% NaCl), resuspended at the desired concentration in saline, and irradiated. Irradiation by UV light (260 nm) or by γ-rays (^{60}Co) was performed as described earlier (1). For mutagenesis, cells were treated after plating.

Mutation and recombination rates. In each experiment, 12 independent cultures inoculated at 10^2/ml in liquid YPD medium were grown for 72 h up to the stationary phase. For each one, the frequency of recombinants (His^+) or mutants (*can1* or Lys^+) was determined by plating on differential medium. The rates were determined by the method of the medium (31). The meiotic rates of recombination were measured by treating sporulated cultures with Zymolyase (Seikagaku Kogyo Co. Ltd.) for 4 h, a treatment that kills most of the vegetative cells and digests the walls of asci. After the cultures were washed and sonicated, aliquots were plated on complete or selective medium to determine the frequencies of His^+ clones. To show that the clones were derived from asci and not from vegetative cells, the frequencies of clones in which the recessive markers had segregated out were determined by replica plating.

DNA manipulations and sequencing. All the DNA manipulation and sequencing methods used were the standard ones described by Maniatis et al. (34). For sequencing, the *Bam*HI fragment (see Fig. 6) was subcloned into pTZ18R and pTZ19R plasmids and overlapping deletions were obtained by DNase I treatments (32). Sequencing was done by the dideoxy technique (46).

***lacZ* fusion and *lacZ* assays.** *lacZ* was fused to *RAD51* using a mini-Mu derivative transposon containing the yeast *LEU2* gene and the 2μm circle origin of replication (10). The *Bam*HI-*Eco*RV fragment containing *RAD51* was cloned into pBR322, and the resultant plasmid was used to transform a

bacterial strain containing the inducible transposon. Plasmids of transductants that expressed *lacZ* (tested on LB medium supplemented with 20 μg of X-Gal [5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside] per ml) were extracted and subjected to restriction analysis. Plasmid p51G-1 with an in-frame *RAD51-lacZ* fusion close to the *Nru*I site (see Fig. 6) was integrated into the *RAD51* locus of the FF18733 haploids. Quantification of β-galactosidase by colorimetry using chlorophenol red–β-D-galactopyranoside as the substrate was done by the method of Simon and Lis (50) and performed on aliquots of liquid cultures containing 5×10^6 to 2×10^7 cells. β-Galactosidase units correspond to $(OD_{574} \times 1{,}000)$/minutes of reaction/$(OD_{660} \times V)$, where OD_{574} is the optical density at 574 nm and V is the volume (in milliliters) of the aliquot.

Nucleotide sequence accession number. The *RAD51* sequence reported in this article has been registered in the EMBL data library and given accession number X64270.

RESULTS

Isolation and characterization of suppressors of the radiosensitivity of *srs2::LEU2/srs2::LEU2* diploids. (i) Isolation of the suppressed strains. Diploids homozygous for a genomic deletion of *SRS2* are sensitive to the lethal effects of UV, ionizing radiation, and MMS (1). To select suppressors of MMS sensitivity, colonies of strain FF18749, homozygous for *srs2::LEU2*, were replica plated on YPD medium containing MMS at a concentration that inhibits growth of the mutant but not that of wild-type cells. Since MMS is a radiomimetic drug, the resistant cells were expected to have also become γ-ray resistant. After 4 days of incubation, a number of papillae developed into colonies, of which 11 were analyzed. All of the mutations had similar effects and were found to affect a single gene. We therefore describe only one of them.

(ii) Meiotic analysis of the suppressed *srs2::LEU2/srs2::LEU2* diploids. An MMS-resistant clone (FF18784) isolated from the sensitive *srs2::LEU2* homozygous diploids (FF18749), was sporulated and 24 asci were dissected. Of the 24 asci, 22 had 4 viable spores, indicating that not only the MMS sensitivity but also the defect in spore germination conferred by the *srs2::LEU2* deletion was suppressed (about 40% inviable spores for *srs2::LEU2* homozygotes versus less than 5% for isogenic wild-type cells).

In all tetrads, the MMS resistance segregated 2:2. Since the haploid *srs2::LEU2* cells are MMS resistant, this suggested that the sensitive clones were those containing the suppressor which made the *srs2::LEU2/srs2::LEU2* diploids resistant. To see whether this was indeed the case, the tetrads were crossed with *srs2::LEU2* tester strains. The MMS resistance of the resultant diploids segregated 2:2, and the resistant diploids (*srs2::LEU2/srs2::LEU2 SUP/sup*) were those which had the MMS-sensitive cells (*srs2::LEU2 SUP*) as parental haploids. Thus, the initial diploid contained a single mutation which in the heterozygous state renders *srs2::LEU2/srs2::LEU2* diploids resistant to MMS and *srs2::LEU2* haploids sensitive to MMS.

(iii) Isolation of the suppressor from the *srs2::LEU2* context. A *srs2::LEU2* haploid strain (FF18785) containing the suppressor mutation was crossed with isogenic wild-type cells (FF18734). The diploids were sporulated and asci were dissected in order to separate the suppressor mutation from *srs2::LEU2*. The MMS sensitivity segregated 2:2, independently from the segregation of *srs2::LEU2*, indicating that the mutation by itself confers MMS sensitivity. Qualitative tests of UV sensitivity revealed that this mutation had no sensitizing effect. It suggested that the mutated gene could be one of the genes of the *RAD50* series, since haploid *rad50* mutants are MMS sensitive and only weakly UV sensitive.

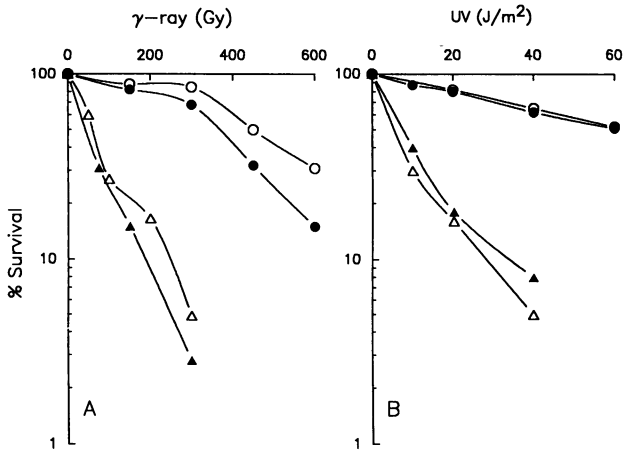

FIG. 1. Suppression of the sensitivity of *srs2::LEU2* homozygous diploids by the heterozygous *rad51-10* mutation. Symbols: ○, *SRS2/SRS2 RAD51/RAD51* (FF18735); ○, *srs2::LEU2/srs2::LEU2 rad51-10/RAD51* (FF18784); △, *srs2::LEU2/srs2::LEU2 RAD51/RAD51* (FF18749); ▲, *srs2::LEU2/srs2::LEU2 rad51::URA3/RAD51* (FF18952).

(iv) The suppressor is localized to *RAD51*. Complementation tests for MMS sensitivity between our suppressor mutant and *rad50* to *rad57* mutants were performed. No complementation was found with a *rad51-1* strain isolated on the basis of X-ray sensitivity. We then crossed our mutant (FF18787) with a *met6* mutant (FF18588). *MET6* is tightly linked to *RAD51*. Of 42 tetrads, no recombination between *met6* and the suppressor was found, indicating that the mutation was most likely in *RAD51*. The definitive proof was later obtained after cloning of the gene, by plasmid complementation of *rad51* mutants. We named these suppressors *rad51-10* to *rad51-20*.

Genetic and physiological properties conferred by *rad51-10*. (i) Effects of *rad51-10* in *srs2::LEU2* cells: codominance of the mutation. The cloning of the *RAD51* gene allowed the construction of strains with chromosomal deletions of this gene (described below). The UV and γ-ray survival of isogenic strains with different combinations of *rad51-10*, *rad51::URA3*, *srs2::LEU2*, and the corresponding wild-type genes were compared. Since *rad51-10* was found to have a dominant suppressor effect on the MMS sensitivity of *srs2::LEU2* cells, we questioned whether this effect was specific to the *rad51-10* mutation or related to the absence of functional Rad51 protein.

Diploids homozygous for *srs2::LEU2* and heterozygous for *rad51-10* show survival rates close to that of wild-type cells, after exposure to γ-rays or UV (Fig. 1). In contrast, heterozygosity for *rad51::URA3* (Fig. 1) or *rad51-1* (data not shown) does not suppress the UV or γ-ray sensitivity of diploids homozygous for *srs2::LEU2* (Fig. 1). The dominant suppressor effect of *rad51-10* is therefore specific to this mutation.

When the *rad51-10* or *rad51::URA3* mutation is homozygous, the cells are still γ-ray sensitive, but their UV sensitivity is largely suppressed (Fig. 2). This suppression is, however, not specific to *rad51* mutants, since mutations in

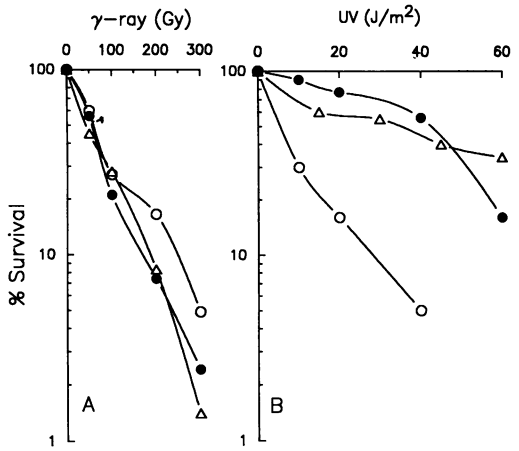

FIG. 2. Radiation survival of diploids homozygous for *srs2*::*LEU2* and for *rad51* mutations. Symbols: ●, *srs2*::*LEU2*/*srs2*::*LEU2 rad51-10*/*rad51-10* (FF18789); △, *srs2*::*LEU2*/*srs2*::*LEU2 rad51*::*URA3 rad51*::*URA3* (FF18963); ○, *srs2*::*LEU2*/*srs2*::*LEU2 RAD51*/*RAD51* (FF18749).

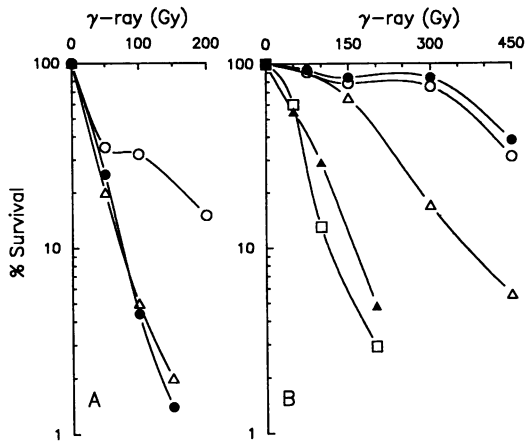

FIG. 4. γ-ray survival of *SRS2* haploids and homozygous *SRS2* diploids containing different *rad51* mutations. (A) Haploids. Symbols: ○, *RAD51* (FF18733); ●, *rad51-10* (FF18816); △, *rad51*::*URA3* (FF18958). (B) Diploids. Symbols: ○, *RAD51*/*RAD51* (FF18735); ●, *rad51*::*URA3*/*RAD51* (FF181048); Δ, *rad51-10*/*RAD51 (FF18981);* ▲, *rad51-10*/*rad51-10* (FF18982); □, *rad51*::*URA3*/*rad51*::*URA3* (FF18960).

other genes involved in recombinational repair have the same effect (unpublished results).

In haploids, as shown in Fig. 3A, the *srs2*::*LEU2* mutants are not more γ-ray sensitive than wild-type cells. The tails of the survival curves, as a result of recombinational repair between sister chromatids in G_2 cells, are eliminated by *rad51-10* as well as by *rad51*::*URA3* mutations.

After UV irradiation (Fig. 3B), the *srs2*::*LEU2* cells are sensitive if irradiated in G_1 but not if cells are irradiated in G_2. The lack of G_1 repair was shown to be correlated with a deficiency in error-prone repair, explained by a channelling of the metabolism of potentially mutagenic lesions into a recombinational pathway (1). The UV sensitization by *srs2*::*LEU2* of G_1 haploids is suppressed not only by *rad51-10* but also by *rad51*::*URA3*. At the same time, induced mutagenesis is restored, as shown in Fig. 3B for *rad51-10*. A similar suppression was found to be conferred by mutations in other genes governing recombination (unpublished results).

These data show that *rad51-10* suppresses the *srs2*::*LEU2* defect and at the same time eliminates recombinational repair. The fact that *srs2*::*LEU2*/*srs2*::*LEU2* diploids, heterozygous for *rad51-10*, have almost wild-type phenotypes after exposure to γ-rays or UV indicates that both the wild-type and mutated genes are expressed: *RAD51* allows recombinational repair to occur, and *rad51-10* suppresses the *srs2*::*LEU2* effects. The two allelic forms are therefore codominant.

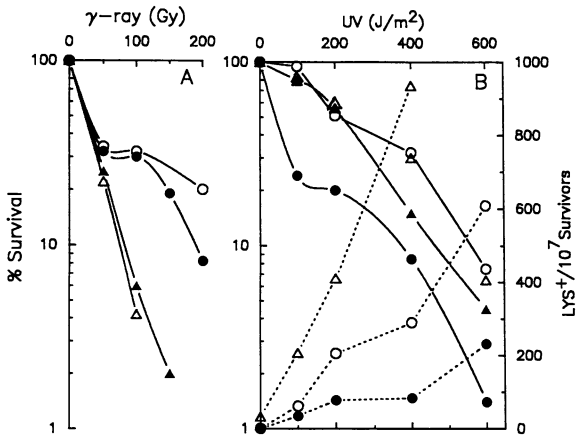

FIG. 3. Radiation responses of haploid cells. Symbols: ○, *SRS2 RAD51* (FF18733); ○, *srs2*::*LEU2 RAD51* (FF18744); △, *srs2*::*LEU2 rad51-10* (FF18785); ▲, *srs2*::*LEU2 rad51*::*URA3* (FF18961).

(ii) Effects of *rad51-10* in *SRS2*$^+$ cells: semidominance of the mutation. In the *SRS2*$^+$ genetic context, *rad51-10* or *rad51*::*URA3* haploids or homozygous diploids are only weakly (if at all) UV sensitive (data not shown) but are γ-ray sensitive: recombinational repair is eliminated by the mutations, as seen from the lack of repair in haploids in G_2 (Fig. 4A) and from the diploid sensitivity (Fig. 4B). In heterozygous diploids, the *rad51*::*URA3* mutation is recessive, while the *rad51-10* mutation is semidominant: these diploids are significantly more sensitive than wild-type diploids but are still much more resistant than homozygous *rad51*/*rad51* cells. This semidominance of *rad51-10* was also observed for sporulation. Sporulation of homozygous *rad51-10* diploids was 10% (versus 70% for wild-type cells) and spore viability was about 2% (2 viable monosporic clones of 24 tetrads), versus 96% for wild-type cells. In heterozygous *rad51-10* cells, sporulation was 30% and spore viability was 60%.

In order to determine whether the semidominance of *rad51-10* was related to the relative proportions of the wild-type and mutated proteins, a series of isogenic tetraploids containing different numbers of *rad51-10* alleles was constructed. The γ-ray sensitivity of the cells increases with the copy number of the mutated allele. After 200 Gy, the values for percent survival were 50, 13, 7, 1.2, and 0.9 for tetraploids containing 0, 1, 2, 3, or 4 copies of *rad51-10*, respectively. Since the recessiveness of *rad51*::*URA3* indicates that the Rad51 protein is not rate limiting, this gene dosage effect suggests that the Rad51-10 protein competes with the wild-type Rad51 protein either in protein-protein interactions or in the binding to recombinational structures.

(iii) Mutator effect of *rad51-10*. The rates of spontaneous forward mutations leading to canavanine resistance

TABLE 2. UV and γ-ray induction of His$^+$ revertants in heteroallelic diploids[a]

Relevant genotype	Revertants per 10^6 survivors and radiation survival (%)									
	γ-rays (Gy)					UV (J/m^2)				
	0	50	100	150	200	0	15	30	45	60
RAD51/RAD51	4 (100)	41 (80)	88 (70)	102 (61)	182 (49)	3 (100)	33 (100)	90 (100)	190 (95)	387 (73)
rad51::URA3/rad51::URA3	0.3 (100)	<0.7 (45)	1.3 (24)	<1 (7)	NT[b]	0.5 (100)	1.7 (87)	1.3 (74)	10 (34)	4 (14)
rad51-10/rad51-10	<0.1 (100)	0.25 (58)	0.2 (22)	NT	<2 (7)	0.15 (100)	1.3 (92)	2.5 (64)	4.5 (58)	16 (20)
rad51-10/RAD51	0.2 (100)	30 (54)	61 (50)	92 (31)	139 (20)	0.5 (100)	5 (71)	16 (57)	45 (50)	106 (36)
rad51::URA3/RAD51	1.3 (100)	33 (90)	75 (78)	130 (65)	140 (53)	0.8 (100)	27 (100)	85 (100)	167 (85)	348 (68)

[a] The heteroallelic *his7-1/his7-2* strains FF181002 (*RAD51/RAD51*), FF181005 (*rad51::URA3/rad51::URA3*), and FF181173 (*rad51::URA3/RAD51*) are isogenic. Strains FF18056 (*rad51-10/rad51-10*) and FF181174 (*rad51-10/RAD51*) have a different genetic background but are isogenic.
[b] NT, not tested.

(*CAN→can1*) were determined by fluctuation tests (see Materials and Methods). For *rad51-10* (FF18816) and *rad51::URA3* (FF18958) cells, the rates were found to increase by a factor of at least 10 (42 and 51 per 10^7 cell divisions, respectively) compared with the rate (3.8 per 10^7) in wild-type cells (FF18733). Such a mutator effect has been previously reported for other *rad51* mutants and one of them, *rad51-3* (*mut5*) was even isolated as a mutator strain (18).

(iv) Effects of *rad51-10* on intragenic recombination. The rates of spontaneous His$^+$ reversion events were determined by fluctuation tests, in diploids heteroallelic for *his7* and with different combinations of *RAD51*, *rad51-10*, or *rad51::URA3* alleles. In *rad51-10* (FF181056) or *rad51::URA3* (FF181005) homozygotes, the rates found, in both strains, were 0.2 per 10^7 cell divisions, i.e., a 20-fold decrease compared with the rate (4 per 10^7) found for *RAD51* homozygotes (FF181002). In view of the mutator phenotype of *rad51* mutants, it is possible that the histidine prototrophs were formed by mutagenesis and not by recombination. In the heterozygous state, *rad51-10* had a semidominant effect, the rate (0.9 per 10^7) being decreased by a factor of 4 with respect to the wild-type control.

After UV or γ-ray treatments (Table 2), His$^+$ inductions in homozygous *rad51* mutants were considerably decreased and, as mentioned above, the reversions were possibly due to mutation rather than to recombination events. In heterozygous cells, *rad51::URA3* was found to be recessive while *rad51-10* was semidominant, but only for UV induction. This, we believe, reflects a channelling of the metabolism of potentially recombinogenic UV lesions into another pathway.

In summary, these results show that *rad51-10* and *rad51::URA3* mutations considerably reduce, if not abolish, spontaneous and induced intragenic recombination, as it has been shown for other *rad51* mutations (36, 45). They also show that spontaneous or UV-induced lesions that initiate recombination in wild-type cells are in the absence of the *RAD51*-encoded function or in the presence of the semidominant *rad51-10* allele channelled into a nonrecombinogenic process.

(v) Mating-type switching. The mating-type switch is initiated by a double-strand break made by the HO endonuclease in the *MAT* locus. This break is repaired by recombination with a silent *MAT* copy residing at the *HML* or *HMR* locus, generating a switch of the mating type (for a review, see reference 53). After sporulation of diploids heterozygous for *HO*, the monosporic clones containing *HO* become diploid as a result of mating between cells of opposite mating type. The combination of *HO* and another mutation preventing the repair of the double-stand breaks in a spore kills the cells. Thus, *rad52 HO* spores do not give rise to viable clones (33). Game (14) reported that some *rad51* mutants were defective in mating-type switching. We asked whether the semidominant alleles and the deletion mutations have the same effect. Diploids heterozygous for *HO* and for *rad51-10* or *rad51::URA3* were constructed by crossing spores of strain DM3878 6B, containing *HO*, with either *rad51-10* or *rad51::URA3* haploids (FF18818 or FF18959). Twenty-four tetrads of each diploid were analyzed. None of the spores, which, by deduction from the analysis of the viable monosporic clones were expected to carry *HO rad51-10* or *HO rad51::URA3*, formed viable clones, indicating that *rad51-10* and *rad51::URA3* prevent the repair of the *HO*-mediated double-strand break.

(vi) Effects of *rad51-10* and *rad51::URA3* on meiotic intragenic recombination. Homozygosity for *rad51-10* considerably reduces sporulation and spore viability (see above). This was also observed for *rad51::URA3* cells (data not shown) and is known for other *rad51* mutants. It is, however, possible to study the levels of meiotic recombination among viable spores (see Material and Methods). In strains that were heteroallelic for *his7*, we found that the rates of meiotic formation of histidine prototrophs were reduced by a factor of 20 in cells homozygous for *rad51-10* (FF181056) or *rad51::URA3* (FF181005) compared with the wild-type (FF181002) rate. The values found were 4, 5, and 90 histidine prototrophs per 10^5 viable spores, respectively.

Isolation of *RAD51*, similarities of the putative protein with RecA, and construction of a genomic deletion. (i) Cloning of *RAD51*. The *RAD51* gene was cloned by complementation of the MMS sensitivity of *rad51-10* cells. Diploids (FF18982) homozygous for this mutation were transformed with a centromeric YCp50 (*ARS1 CEN4 URA3*) (25) yeast genomic bank (43). Among 3,000 Ura$^+$ transformants studied, one was MMS resistant and sporulated efficiently. These phenotypes cosegregated with the plasmid. The plasmid was extracted and amplified in *E. coli*. It contained a 17-kb insert which was subcloned into YRp7 (*ARS1-TRP1*) vector (55). A *Bam*HI fragment of 3.7 kb was found to complement *rad51-1* and *rad51-10* mutations. The restriction map is shown in Fig. 5. This plasmid, after digestion by *Hpa*I which recognizes a unique site in the insert, was integrated into diploid strain FF18981 which is homozygous for *trp1* and heterozygous for *rad51-10*. Two transformants were subjected to meiotic analysis. In each case, no recombination between *TRP1* and *rad51* occurred among 24 tetrads analyzed. This was the result expected if the insert contained the *RAD51* gene.

(ii) Nucleotide and amino acid sequences. The 3.7-kb *Bam*HI fragment (Fig. 5) was sequenced. It was found to

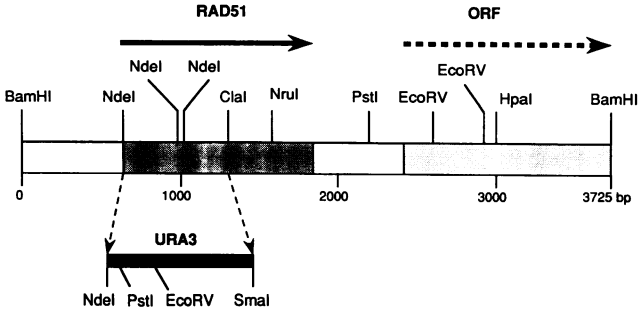

FIG. 5. Restriction maps of the genomic *Bam*HI fragment containing the *RAD51* gene or the *rad51*::*URA3* construction. The *Sma*I and *Cla*I termini were made blunt before ligation.

contain two open reading frames (ORFs), of 1,200 and 1,302 bp, respectively, in the same orientation and separated by 515 bp. The second ORF is truncated. The *Bam*HI-*Eco*RV fragment was subcloned and found to complement *rad51*, indicating that the first ORF contained *RAD51*.

The sequence of the first 2,174 nucleotides is shown in Fig. 6. The encoded predicted protein is 400-amino-acids long and has a calculated molecular mass of 42,970 Da. It contains ATP/GTP binding domains (21, 57) but no other characteristic consensus domains. It has two acidic regions: among the first 96 amino acids of the N-terminal region, 18 are acidic and 2 are basic. In the C-terminal region, of 33 amino acids, 10 are acidic and 2 are basic. The 5′ upstream region contains two *Mlu*I restriction sites at −154 and −196 bp from the first ATG. Such sites are known to be potential cell cycle-dependent regulatory signals (for a review, see

```
                                                                  BamH1
-644                                                              GGATCCGACATTTT

-630 TTTTTTATGCTTTATTCACTGTTCAATATTTTCACCACAATTCGCAAGAAACGCACTCTACTTCGAAACTACGGTTCAAACTTACTTAGC

-540 AGCTTCCCGATTTAATTGGCCTTTCTACTATGCCATAAACTCTTTCTTCCTCTCTTTTCATCGCCCCTGCATTTGCACTTTTTTGCCACC

-450 GGCAGTGCCATCCGGTCACATGACTACACCACGTTAATAGCGATCTGGCTTATCATTGTCACAGAGTAAATTAAAATGGACGGTAAATGT

-360 TGGAAATGCACCACTACCGTTCTTCAACCAATCTAGTTTAGCTATCCTGCAACAGGTGGCCTTCTTGAGCATTCCCTGAGCATTCCAACC

-270 GGTTGTATCAGTGTTTTATCACCGTCTCACCATATCCCACGACTAGGCCACACTTCGTTACCCTATGCTACGCGTCATTTCCGCTATTTC

-180 TGTCCTGGTTTGTTTACAGTACGCGTGGTGGGACCATAAAGGGGAATAGTGGGGACTGGAGAAAAAATTTTCTCAGTTACTTCTTCTATC

-90  TTCCGTAGTTTCCATATACTAGTAGTTGAGTGTAGCGACAAAGAGCAGACGTAGTTATTTGTTAAAGGCCTACTAATTTGTTATCGTCAT

+1   M  S  Q  V  Q  E  Q  H  I  S  E  S  Q  L  Q  Y  G  N  G  S  L  M  S  T  V  P  A  D  L  S
+1   ATGTCTCAAGTTCAAGAACAACATATATCAGAGTCACAGCTTCAGTACGGGAACGGTTCGTTGATGTCCACTGTACCAGCAGACCTTTCA

31   Q  S  V  V  D  G  N  G  N  G  S  S  E  D  I  E  A  T  N  G  S  G  D  G  G  G  L  Q  E  Q
91   CAGTCAGTCGTTGATGGAAACGGCAACGGTAGCAGCGAAGATATTGAGGCCACCAACGGCTCCGGCGATGGTGGCGGATTGCAGGAGCAA

61   A  E  A  Q  G  E  M  E  D  E  A  Y  D  E  A  A  L  G  S  F  V  P  I  E  K  L  Q  V  N  G
181  GCGGAAGCGCAAGGTGAAATGGAGGATGAAGCATACGATGAAGCTGCCTTAGGTTCGTTTGTGCCAATAGAAAAACTGCAAGTGAACGGG

91   I  T  M  A  D  V  K  K  L  R  E  S  G  L  H  T  A  E  A  V  A  Y  A  P  R  K  D  L  L  E
270  ATTACTATGGCGGATGTGAAAAAACTAAGGGAGAGTGGGCTTCACACTGCTGAAGCGGTAGCATATGCTCCCAGAAAGGATTTATTGGAA

121  I  K  G  I  S  E  A  K  A  D  K  L  L  N  E  A  A  R  L  V  P  M  G  F  V  T  A  A  D  F
361  ATCAAAGGTATATCGGAAGCTAAGGCAGATAAGTTGCTAAACGAAGCGGCAAGGCTAGTGCCTATGGGATTTGTCACGGCTGCTGATTTT

151  H  M  R  R  S  E  L  I  C  L  T  T  G  S  K  N  L  D  T  L  L  G  G  G  V  E  T  G  S  I
451  CATATGAGAAGATCGGAGCTGATTTGTTTGACAACGGGTTCTAAGAATTTGGACACTCTTTTGGGTGGTGGTGTGGAAACTGGTTCTATT

181  T  E  L  F  G  E  F  R  T  G  K  S  Q  L  C  H  T  L  A  V  T  C  Q  I  P  L  D  I  G  G
541  ACTGAGCTTTTCGGTGAATTCAGGACAGGTAAGTCCCAGCTATGTCACACTTTGGCCGTGACATGCCAAATTCCATTGGATATTGGTGGC

211  G  E  G  K  C  L  Y  I  D  T  E  G  T  F  R  P  V  R  L  V  S  I  A  Q  R  F  G  L  D  P
631  GGTGAAGGTAAGTGTTTGTATATCGATACCGAAGGTACTTTCAGGCCGGTAAGATTGGTATCCATAGCTCAGCGGTTCGGATTAGACCCG

241  D  D  A  L  N  N  V  A  Y  A  R  A  Y  N  A  D  H  Q  L  R  L  L  D  A  A  A  Q  M  M  S
721  GATGATGCTTTGAACAACGTTGCGTATGCAAGAGCCTATAACGCCGATCATCAGTTAAGACTTCTGGATGCTGCTGCCCAAATGATGAGC

271  E  S  R  F  S  L  I  V  Y  D  S  V  M  A  L  Y  R  T  D  F  S  G  R  G  E  L  S  A  R  Q
811  GAGTCTCGGTTTTCCTTGATTGTGGTCGATTCTGTTATGGCTCTATACCGTACGGATTTTTCTGGTCGTGGTGAACTAAGCGCAAGGCAA

301  M  H  L  A  K  F  M  R  A  L  Q  R  L  A  D  Q  F  G  V  A  V  V  V  T  N  Q  V  V  A  Q
901  ATGCATTTAGCCAAATTTATGCGTGCTTTGCAAAGGCTGGCCGACCAATTTGGTGTTGCAGTCGTCGTTACTAACCAAGTGGTCGCCCAA

331  V  D  G  G  M  A  F  N  P  D  P  K  K  P  I  G  G  N  I  M  A  H  S  S  T  T  R  L  G  F
991  GTTGATGGTGGTATGGCTTTTAATCCAGATCCAAAGAAGCCTATCGGTGGTAATATTATGGCACATTCTTCCACCACGCGATTAGGTTTC

361  K  K  G  K  G  C  Q  R  L  C  K  V  V  D  S  P  C  L  P  E  A  E  C  V  F  A  I  Y  E  D
1081 AAAAAGGGTAAGGGATGTCAAAGATTATGCAAAGTTGTTGACTCACCTTGCTTACCAGAGGCTGAATGTGTGTTCGCGATCTATGAAGAT

391  G  V  G  D  P  R  E  E  D  E  •
1171 GGTGTTGGTGACCCCAGAGAAGAAGACGAGTAGGTATTTGGTCTCTTGTCTCTATTTATTTACACAGGTTTACTTTCAATTCTCCTCTTT

1261 TTCTTAGGTTGCGTTCCGTACATTTTTATCTTCATTTCCATCCACTGTCTTAGATTTTTGCATATATTTTGTCATATACCTCGCAACCCT

1351 ACTGCGGGTCTTAACCTTTTTTTTCAGTTCTTTTAAATAACTTTCGTATTGTCTGTCACCCATGAAATATATGTATTTTTCTACTCTTCT

1441 TCCCGATGACTACTTCCTCCTGCAGGGTCCGCGCGGCTTTATCCTTTTAGGGGAGTGAAGAGAAAAATTTTCTGATATGTCGCCATCCTC
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FIG. 6. Sequence of the *RAD51* gene and flanking DNA. The predicted amino acid sequence is shown by the one-letter code. Nucleotide residues are numbered relative to the ATG that initiates the ORF. The termination codon is indicated by a solid circle. Underlined regions at the 5′ end indicate the *Mlu*I restriction sites, and underlined regions at the 3′ end indicate putative transcription termination signals. The nucleotide binding consensus sequence GXXXXGKS and hhhhD (where h are hydrophobic amino acids) are stippled.

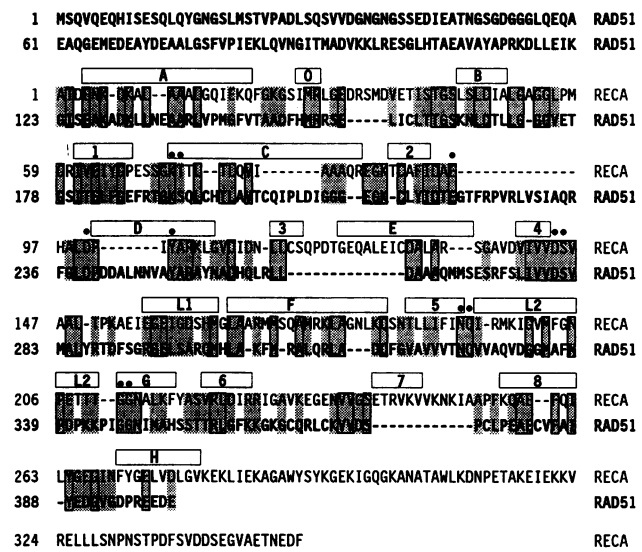

FIG. 7. Amino acid sequence homology between the Rad51 and the *E. coli* RecA proteins. Identical and conservatively substituted amino acids are boxed and shaded or shaded, respectively. RecA domains are indicated as defined by Story et al. (52). β-strands are indicated by numbers and α-helices are indicated by letters from A to H. L1 and L2 are disordered loops. Solid circles indicate invariant or conservatively substituted amino acid residues found in procaryotic RecA and T4 UvsX proteins and involved in ATP binding, ATP hydrolysis, and/or conformational changes (51).

reference 3). Sequences downstream of the translation stop codon show similarities to yeast transcription signals at distances from the stop codon comparable to those observed in the corresponding *CYC1* region (58).

(iii) Deduced Rad51 protein shows similarity with the procaryotic RecA proteins. A computer search for homologies with other proteins, using the FASTA research program (40), revealed that the highest homology scores were with the procaryotic RecA proteins. Figure 7 shows a possible alignment of the *E. coli* RecA (22) and Rad51 proteins. Although the two proteins have comparable sizes (352 and 400 amino acids, respectively), the region of similarities (see reference 6 for similarity matrix) do not overlap the whole proteins. The N-terminal region of Rad51 (122 amino acids) has no significant homology with RecA, and the C-terminal region of RecA (76 amino acids) has no significant homology with Rad51. In the overlapping region, if one excludes the gaps introduced in either sequence, 38% of residues are identical and 29% are similar. The relation between the structure of *E. coli* RecA protein and the properties of numerous *recA* mutants and mutant proteins allowed Story et al. (51, 52) to propose a correspondence between the protein domains and the different activities of the protein. In Fig. 7 are indicated the RecA domains, as they are defined by Story et al. (52), allowing to predict by sequence comparison the corresponding domains of Rad51 and their respective roles. Significant homologies are found with domains predicted to be involved in the following: (i) monomer interaction to form polymers (regions A and 0 of RecA); (ii) single-stranded DNA binding (regions L2 and G); (iii) double-stranded DNA binding (region L1); and (iv) ATP binding, ATP hydrolysis, and conformational changes of the protein. Amino acid residues, which in the model play a key role in these reactions and which are invariant or conservatively substituted in procaryotic RecA and T4 UvsX proteins (for a review, see reference 41) are also conserved in Rad51. These residues, shown in the one-letter code and with reference to the *E. coli* protein, are as follows: Y-103, D-100, and G-265 (E in Rad51), involved in adenine fixation; K-72 and T-73 (S in Rad51), involved in interactions with the β- and γ-phosphate of ATP; D-144, S-145, and E-96, involved in ATP hydrolysis; and N-193, Q-194, G-211, and G-212 involved in conformational changes upon ATP hydrolysis. Regions of RecA with no homology to Rad51 include the C-terminal part proposed to be involved in polymer interaction, β-strand region 7, part of the following loop (242 to 252) that is predicted to interact with LexA repressor, β-strand region 3, and α-helix E that interacts with the A and 0 region of another RecA molecule. The Rad51 protein has also regions of nonhomology to RecA, including its long N-terminal tail. Obviously, although the two proteins differ in many respects, the ATP binding core of the protein and the DNA binding regions appear to be conserved.

(iv) Construction of genomic deletion of *RAD51*. The *Nde*I-*Cla*I fragment of the *RAD51* clone was replaced by the *Nde*I-*Sma*I fragment of YIp5 containing the *URA3* gene (Fig. 5), thus deleting the sequence coding for the 184 N-terminal amino acids of Rad51. This plasmid (pTZ51Δ) was cut by *Bam*HI and used to transform *ura3* cells in order to disrupt the wild-type resident gene. The transformants were analyzed for MMS sensitivity. Southern analysis of the genomic DNA of the MMS-sensitive transformants showed that in all cases, the chromosomal rearrangements corresponded to the expected gene replacement event.

Inducibility of *RAD51* by DNA-damaging agents. A plasmid containing a *RAD51-lacZ* gene fusion was constructed by transposition in *RAD51* of a mini-Mu containing *lacZ*, the yeast *LEU2* gene, and the 2μm origin of replication (10). This plasmid was integrated into the *RAD51* locus of haploid cells. These cells therefore contain a wild-type copy of *RAD51* and the chimeric gene under the control of the *RAD51* promoter. They have a wild-type phenotype. Cells in the logarithmic phase were irradiated and reincubated in YPD medium, and the β-galactosidase activity was measured in aliquots after different times of incubation. These experiments will be reported in more detail elsewhere; we show here the results of a typical experiment with cells having received UV doses of 0, 5, or 50 J/m^2 (Fig. 8). Comparable results were obtained after γ-ray irradiation. The β-galactosidase activity increased rapidly following irradiation, peaking after 1.5 and 2.5 h of incubation. Upon further incubation, the activity progressively declined, reaching the level seen in the untreated cells after 8 h. The maximal induction was dose dependent, indicating that the level of induction is likely to depend on the amount of damage induced in the cells. Northern (RNA) analysis of the mRNA by G. Basile (4) and by T. Ogawa (38) also demonstrated the inducibility of *RAD51*.

DISCUSSION

In this report, we describe the isolation and characterization of suppressors of the UV and γ-ray sensitivities of diploids homozygous for a genomic deletion of *SRS2*, a gene coding for a DNA helicase (1). In all cases, the suppression was found to be due to an heterozygous mutation in *RAD51*, a gene involved in recombinational repair. These new *rad51* mutations eliminate recombinational repair but differ from other mutations, including the deletion, in that they suppress in the heterozygous state the phenotypes of *srs2*::*LEU2* homozygous cells and are, by themselves, semidominant.

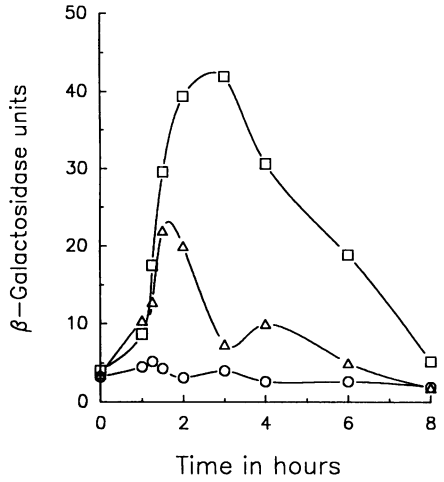

FIG. 8. Expression of *RAD51* following UV irradiation. Aliquots (10 ml) of a culture of exponentially growing cells containing a *rad51-lacZ* fusion (FF181082) were filtered. The cells were resuspended in saline to be irradiated after they were filtered and resuspended in YPD medium (10^7/ml). Duplicate aliquots (1 ml) were taken after different times of reincubation at 30°C for β-galactosidase assays. The UV doses applied were 0 (○), 5 (△), and 50 (□) J/m^2.

TABLE 3. UV and γ-ray sensitivities of haploids and diploids in G_1 and G_2 mitotic phases

Cell type and relevant genotype	Sensitivity to treatment[a]			
	UV		γ-rays	
	G_1	G_2	G_1	G_2
Haploids				
RAD51	+	+	−	+
srs2::LEU2	−	+	−	+
rad51-10	+	+	−	−
srs2::LEU2 rad51-10	+	+	−	−
rad51::URA3	+	+	−	−
srs2::LEU2 rad51::URA3	+	+	−	−
Diploids				
RAD51/RAD51	+	+	+	+
srs2::LEU2/srs2::LEU2	−	+−	−	+−
rad51-10/rad51-10	+	+	−	−
srs2::LEU2/srs2::LEU2 rad51-10/rad51-10	+	+	−	−
rad51::URA3/rad51::URA3	+	+	−	−
rad51-10/rad51-10	+	+	−	−
srs2::LEU2/srs2::LEU2 rad51-10/RAD51	+	+	+	+
srs2::LEU2/srs2::LEU2 rad51::URA3/RAD51	−	+−	−	+−
rad51-10/RAD51	+	+	+−	+−
rad51::URA3/RAD51	+	+	+	+

[a] +, wild-type sensitivity; −, large increase in sensitivity; +−, slight increase in sensitivity. For the sake of clarity, the increased UV sensitivity of *rad51* versus *RAD51* homozygous diploids is not taken into account.

We also report the sequence of *RAD51*, which reveals similarities between the deduced protein and the procaryotic RecA protein, and preliminary results showing the inducibility of *RAD51* by DNA-damaging agents.

Analysis of the results presented here, together with those earlier published (1), leads us to the following interpretation: the Srs2 helicase is involved in the metabolism of single-strand gaps, preparing a substrate for a DNA polymerase. In the absence of Srs2, recombination proteins bind to these gaps and channel their metabolism into a recombinational pathway. For as yet unknown reasons, recombination repair is successful when the events involve sister chromatids but lethal if they involve homologous chromosomes. A block in recombination prevents this channelling, and although the Srs2 helicase is absent, the gap is filled in by replication.

The above interpretation is partially based on comparisons of UV and γ-ray responses. UV does not induce double-strand breaks, but single-strand gaps are presumably formed when close lesions on opposite strands are induced, the excision of one uncovering the other. Such gaps can be filled by error-prone replication with no involvement of recombination. Thus, UV survival curves of wild-type or recombination-deficient cells are sigmoidal and either do not or very weakly reveal heterogeneity in the cell population. Haploid cells with mutations in some genes of this pathway, such as *SRS2* or *REV3* (which codes for the putative error-prone polymerase [35]), are sensitive to UV if irradiated in the G_1 mitotic phase but resistant in G_2 because of recombinational repair between sister chromatids. In contrast, γ-rays induce double-strand and single-strand breaks, the double-strand breaks being repaired in haploids only by recombination between sister chromatids and in diploids, by exchanges between sister chromatids or homologous chromosomes. Mutants deficient in recombinational repair are γ-ray sensitive and UV resistant. Thus, in some cases, the comparison of UV and γ-ray responses indicates which repair pathways are active in mutant cells, which can be further tested by the study of radiation-induced mutagenesis and recombinogenesis.

Table 3 summarizes the relative radiation sensitivities of the different strains studied here. The *srs2::LEU2* haploids are sensitive to radiation only in G_1. Their G_2 resistance to UV and to γ-rays is high and the UV mutagenesis is depressed: the error-prone repair is deficient, but not the recombinational repair. The *rad51-10* and *rad51::URA3* cells are UV resistant and γ-ray sensitive: the error-prone repair is active, but the recombinational repair is deficient. In double mutants *srs2::LEU2 rad51*, the G_1 UV resistance and mutability are restored, but the cells are now γ-ray sensitive: the blocking of recombination restores the ability of the cells to perform error-prone repair.

The situation is similar in diploids taking into account that following UV or γ-ray irradiation, DNA structures present because of the lack of Srs2 presumably lead to lethal interchromosomal exchanges. It is likely that the γ-ray sensitivity does not result from a deficiency in double-strand break repair, but from the channelling of single-strand gaps, known to occur frequently after γ-ray irradiation, into a recombinational pathway. None of the properties of *srs2::LEU2* cells suggest that they are deficient in double-strand break repair. After UV, a block in recombination results in a suppression of UV sensitivity in diploids and haploids, indicating that single-strand gaps are now repaired. Clearly, this suppression cannot be monitored with γ-rays, since it is due to an absence of recombination which prevents double-strand break repair.

What is unique about the *rad51* mutants that we have isolated as suppressors of the diploid sensitivity of *srs2::LEU2* cells? First, the fact that when selecting MMS-resistant clones from *srs2::LEU2/srs2::LEU2* diploids, we obtained heterozygous mutants for *RAD51* and not for other

genes indicates that this gene plays a key role in this suppression phenomenon. Second, these *rad51* mutants are by themselves semidominant. This is not the case for the *rad51*::*URA3* and *rad51-1* mutations which are recessive and do not suppress, when heterozygous, the *srs2*::*LEU2* sensitivities.

How could *rad51-10*, in the heterozygous state, suppress both the UV and γ-ray sensitivities of *srs2*::*LEU2* cells? Since the haploids derived from these diploids exhibit deficiencies in the error-prone repair process (*srs2*::*LEU2*) or in the recombinational process (*srs2*::*LEU2 rad51-10*), both mechanisms must act in the diploid cells. The *RAD51* and *rad51-10* genes are codominant, *rad51-10* being a dominant suppressor of the defects related to *srs2*::*LEU2* and the wild-type gene conferring a dominant ability to perform recombination. If the UV- or γ-ray-induced structures which are lethal in *srs2*::*LEU2* cells are indeed single-stranded regions, then in the suppressed diploids, Rad51-10, rather than the wild-type protein, binding to these gaps would suppress the lethal channelling. The wild-type protein would be preferentially involved in double-strand break repair. This hypothesis is supported by the fact that the semidominance of *rad51-10* observed in *SRS2*$^+$ diploids is largely abolished in *srs2*::*LEU2* homozygous diploids, as if in the presence of structures caused by *srs2*::*LEU2*, the mutated protein is much less involved in recombinational complexes. An attractive hypothesis is that the Rad51-10 protein has an activity replacing that of Srs2 in the error-prone metabolism. If this is so, it is however not an absolute requirement, since in the deletion mutant, the absence of the Rad51 protein also results in the suppression of the UV sensitivity of *srs2*::*LEU2* cells.

The recombination block in *rad51-10* cells could result from the lack of association of the Rad51-10 protein with other recombination proteins or from the formation of nonfunctional complexes with DNA, as suggested by the semidominance and gene dosage effect of the mutation. By analogy with RecA functions, the *rad51-10* mutation could still allow binding to single-stranded DNA but may prevent polymerization, synapsis formation, or strand exchange. This would explain the different effects of this mutation. There are mutations in *recA*, like *recA1* or *recA142*, which in in vitro and/or in vivo tests, are codominant with the wild-type gene (28, 39). Both of these mutated proteins still bind single-stranded DNA but are unable to promote strand exchange.

The comparison of Rad51 and *E. coli* RecA sequences lends support to the idea that Rad51 protein binds to single-stranded DNA and interacts with itself or with other proteins. *RAD51* and *recA* code for proteins of 400 and 352 amino acids with molecular masses of 43 and 38 kDa, respectively. Regions of homology between these proteins include domains that in RecA, are involved in single-stranded and double-stranded DNA binding, ATP binding, and ATP hydrolysis. These homologies, together with the recombination-defective phenotypes of *rad51* mutants suggest that Rad51 might play the same role in recombination as RecA. Besides these conserved domains, each protein has regions with no homology to the other protein. It is conceivable that these regions are involved in interactions with other proteins. For instance, the RecA domain that is proposed to interact with the LexA repressor has no counterpart in Rad51. It should be kept in mind that even if *recA* controls mutagenesis and recombinogenesis in *E. coli* (for a review, see reference 56), there is no indication for a common control of these two processes in *S. cerevisiae*. Besides its role in recombination, RecA has a central role in mutagenesis, allowing the cleavage of the LexA repressor, thereby inducing a number of different genes. In *S. cerevisiae*, none of the recombination-deficient mutants shows depressed mutagenesis and many mutations, including *rad51*, are mutators. Furthermore, mutants deficient in induced mutagenesis, such as *rad18*, *rad6*, *srs2*::*LEU2*, or *rev3* mutants do not show depressed induced recombination.

Two other genes of *S. cerevisiae* have recently been shown to encode putative proteins sharing homologies with Rad51. One is *RAD57*; *RAD57* mutants are affected in recombinational repair and belong to the same epistatic group as *RAD51* (16). The putative protein (26) has a molecular mass of 52.9 kDa, contains an ATP/GTP binding domain, and was found to share with Rad51 the same regions of similarities to RecA. This raises the question of the respective roles of *RAD51* and *RAD57* in recombination and recombinational repair. The other gene is *DMC1* (5). The corresponding protein shares similarities not only with Rad51 but also with RecA. The expression of *DMC1* is specific to meiosis, and the corresponding protein may have a RecA-like function in meiotic recombination. However, it does not substitute for *RAD51*, since *rad51* homozygous cells sporulate poorly, giving rise to spores with a viability decreased by 1 to 2 orders of magnitude. However, the role of *RAD51* during meiosis is not clear. We found that meiotic intragenic recombination in viable spores was decreased at least 20 times by the homozygous *rad51-10* or *rad51*::*URA3* mutations, while Morrison and Hastings (36) reported that inter- and intragenic meiotic recombination in *rad51-3* (*mut5*) homozygous diploids were at the wild-type level. This phenomenon could be allele dependent.

Two proteins having strand transfer activities have been biochemically characterized in *S. cerevisiae*. One, Stpα (34.8 kDa), is encoded by *DST1* (9, 17). The protein has no significant homologies with RecA. The disrupted mutant cells have decreased rates of meiotic recombination but have no phenotype in mitotic cells. The second one, Sep1 (20, 24), the same protein as Stpβ (11), is encoded by *SEP1* (54), also called *DST2* (12). The protein deduced from the gene sequence has a molecular mass of 175 kDa and also has no significant homologies with other known strand transfer proteins. The studies of disrupted mutants showed that the mutation has pleiotropic effects, including a block in meiosis and slightly decreased rates of spontaneous mitotic recombination. However, the mutants are not sensitive to MMS or γ-rays, indicating that other strand transfer proteins must exist in mitotic cells.

In summary, the results reported here are in agreement with the idea that Rad51 protein, previously known to be involved in recombination, may be a yeast counterpart of the procaryotic RecA protein with respect to its recombination function: (i) Rad51 protein has significant homologies to RecA domains predicted to be involved in DNA binding, ATP binding, and ATP hydrolysis; (ii) the dominant suppression of phenotypes of *srs2*::*LEU2* cells by *rad51-10* is explained by a single-stranded DNA-binding activity; (iii) the semidominance of *rad51-10* and the gene dosage effects demonstrate a competition between the wild-type and mutated proteins, competition that may be due to protein-protein interactions; and (iv) the *RAD51* gene is inducible by DNA-damaging agents.

When preparing this manuscript, we learned that A. Shinohara, H. Ogawa, and T. Ogawa had an article in press (49), describing the *RAD51* sequence and showing that

Rad51 binds to single-stranded DNA, in agreement with the interpretation given to our results.

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